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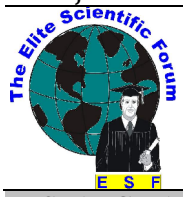

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RESEARCH ARTICLE

Molecular Characterization of Begomovirus Associated Alphasatellite from an Asymptomatic Weed Plant; *Xanthium strumarium* L.

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ABSTRACT
The complete nucleotide sequence of alphasatellite associated with monopartite begomovirus complex isolated from *Xanthium strumarium* L. originating from Pakistan was determined and analyzed. The sequence of alphasatellite showed highest level of nucleotide sequence identity (96.7%) to an isolate of *Gossypium darwinii* symptomless alphasatellite and second highest level of sequence identity (85.8%) identity to an isolate of Papaya leaf curl alphasatellite. Its name was proposed as *Gossypium darwinii* symptomless alphasatellite isolate Xanthium. It is proposed that it can be an isolate of *Gossypium darwinii* symptomless alphasatellite species.

INTRODUCTION

Members of family *Geminiviridae* have single stranded (ss) circular DNA genome and can infect both monocotyledonous as well as dicotyledonous plants. Geminiviruses are transmitted by insect vectors and cause serious threats to many economically important crops throughout tropical and subtropical regions. Based on their host range, genome organization, insect vectors family *Geminiviridae* has been divided into four genera; Mastrevirus, Curtovirus, Topocuvirus and Begomovirus (Brown et al., 2012). Begomovirus is the largest genus of the family which can contains whiteflies transmitted geminiviruses and cause disease in dicotyledonous plants. Begomoviruses which contain two genomic components of size 2.7- 2.8 Kb, namely DNA A and DNA B, called as bipartite while begomovirus with single genomic component equivalent to DNA A component of bipartite are termed as monopartite begomoviruses (Briddon et al., 2008).

In Old World majority of begomoviruses are monopartite and a few are bipartite (Nawaz-ul-Rehman, et al., 2009). They infect cotton, tomato, chili, cassava, papaya etc. Monopartite begomoviruses have recently found associated with ssDNA satellites which influence the etiology of begomoviruses (Briddon et al., 2001, Mansoor et al., 1999). Betasatellite (previously known as DNA β) is ssDNA satellite which is depends on helper begomovirus for replication, encapsidation and movement in the host plant. Betasatellite is almost half in size of helper begomovirus and encode a single protein known as βC1. The product βC1 is pathogenicity determinant and suppressor of RNA silencing (Amin et al., 2011). In presence of betasatellite, begomovirus load is enhanced in the host plant and typical severe disease symptoms appear.

Alphasatellites (previously known as DNA1) is a satellite-like molecule associated with monopartite begomovirus-betasatellite complex. Recent report showed that it can be associated with bipartite begomoviruses (Harimalala et al., 2013). Alphasatellite is almost half in size of helper begomovirus and encode a single protein; replication initiator protein (Rep). Alphasatellite can replicate autonomously but rely on helper virus for encapsidation and movement from cell to cell and long distance movement (Briddon et al.,

2004). It has been observed that in the presence of alphasatellite the level of virus load is decrease as compared to its absence in begomovirus-betasatellite infection (Idris et al., 2011). This feature is antagonistic to that of betasatellite which enhance is the virus level. This may be the strategy of virus etiology for successful infection as well as for host survival. It may also help the virus complex for successful infection by suppressing the host defense mechanism because Rep encoded by alphasatellite also acts as suppressor of RNA silencing (Nawaz-ul-Rehman, 2010).

Begomoviruses are spreading regarding their incidence and geographical distribution. They have emerged as potential threat because of a number of factors including frequent recombination, multiple infections and wide host range. Weeds are involved notoriously in epidemiology of begomovirus because these serve as alternate host as well as recombination pot (Mubin et al., 2012). Weed hosts have reputations for enhanced recombination of begomoviruses (Lima et al., 2012). In present study we have isolated an alphasatellite molecule from an asymptomatic weed plant *Xanthium strumarium* present in cotton field. We sampled the plant as healthy control from the field but hidden enemy being raised was observed. This is first report that begomovirus associated alphasatellite have been characterized from an asymptomatic weed host. Monopartite begomovirus and associated betasatellite from same host showing disease symptoms has been reported by Mubin et al. (2012). This is first report of an alphasatellite from a non symptomatic weed plant *Xanthium strumarium.*

MATERIALS AND METHODS

Leaf sampling and DNA isolation

Leaf samples were collected from *Xanthium strumarium* plants showing symptoms and asymptomatic (Fig. 1A) from suburbs of Dera Ghazi Khan, Punjab Pakistan. Total DNA from asymptomatic weed samples was extracted using CTAB method (Doyle and Doyle, 1990).

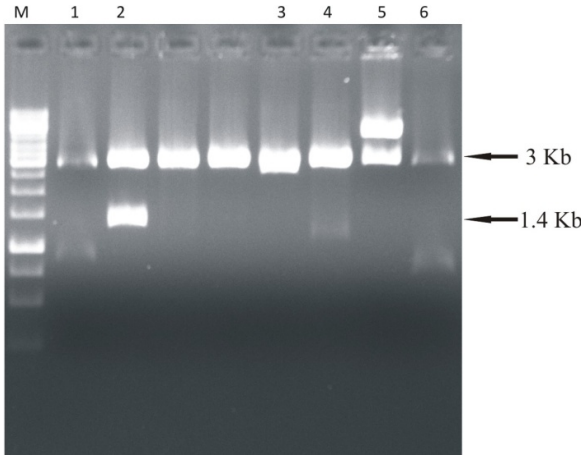
Amplification and cloning of viral components

Rolling circle amplification using phi29 polymerase (Fermentas) was performed for the amplification of circular DNA species and total genomic DNA was used as template. 20 µl reaction mixtures consisted of 5 unit phi29 polymerase, 1 mM dNTPs, 50 µM of random hexamer primers and 1 unit pyrophosphate (as recommended by the manufacturer). After denaturation of template on 94°C for 5 min, the reaction mixture was cooled to room temperature and then phi29 polymerase was added and incubated on 30°C for overnight. Amplified product was restricted with XhoI and cloned into pTZ57R digested with same enzyme (Fermentas).

Table 1: Percentage nucleotide sequence identity between the complete sequences of the DNA of isolate WNSX2 with selected alphasatellites in the database

EU 384637	FN 675303	JQ 322970	JX 262389	NC _005057	NC _005058	NC _013103	NC _014597	NC _015327	NC _015631	NC _018082	AJ 298903	WN SX2	
100	57.4	85.7	66.1	67.1	65.2	62.6	65.1	64.5	56.5	69.6	20.5	96.7	GoDSA (EU384637)
	100	58.6	58.8	58.9	58.6	58.9	58.1	58.1	47	60.5	20.5	57.9	MYMCA (FN675303)
		100	65.5	66.7	66	64.2	66.4	63.8	54	67.9	19.7	85.8	PLCuA (JQ322970)
			100	66.9	66.3	62.7	80.3	70.4	58.2	82.4	19.6	67	CLCuBuA (JX262389)
				100	80.5	72.9	70	69.6	57.4	68.5	19.9	68.8	TCSA (NC 005057)
					100	71.6	66.5	67.1	55.6	69.6	21.4	63.5	ToYLCuCHA (NC 005058)
						100	63.6	64.1	52.4	65.3	20	63.8	ChLCuMA (NC 013103)
							100	72.4	63.5	82.3	20.8	65.8	TLCuPA (NC 014597)
								100	58.9	68.5	19.4	65	CLCuA-Luk (NC 015327)
									100	60	21.2	56.1	VerbLCuA (NC 015631)
										100	18.9	69.7	CLCuMA (NC 018082)
											100	19.9	CLCuMB (AJ298903)
												100	WNSX2

A



B

Fig. 1: A. Field sample of asymptomatic Xanthium strumarium plant B. Confirmation of alphasatellite clone in pTZ57R plasmid vector by restriction analysis using XhoI restriction enzyme.

Sequencing and Sequence analysis

Clones were sequenced completely on both strands using M13 primers by Macrogen (Seoul, Korea). Sequences were assembled and analyzed with the help of DNA star (Lasergene package). Multiple sequence alignments were performed and phylogenetic trees were constructed using Clustal X and trees were displayed and manipulated using Treeview software. Percentage identity figure was determined in MegAlign application of DNAstar. The reference sequences of alphasatellites used in analyses were obtained from databases.

RESULTS AND DISCUSSION

The clone of alphasatellite in pTZ57R (Fermentas) was confirmed by restriction analysis using XhoI enzyme (Fig. 1B). The clone was designated name as WNSX2. The clone was sequenced in its entirety on both

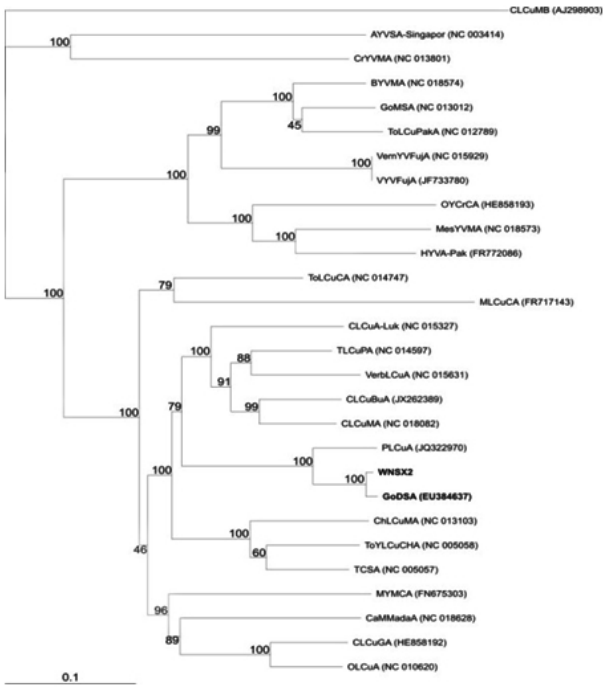


Fig. 2: Phylogenetic dendrogram based on alignments of selected alphasatellite complete DNA sequences.

Vertical branches are arbitrary, horizontal branches are proportional to calculated mutation distances. Values at nodes indicate percentage bootstrap values (1000 replicates). Alphasatellite sequences used for comparison were Cassava mosaic Madagascar alphasatellite (CaMMadaa), Chilli leaf curl Multan alphasatellite (ChLCMA), Cotton leaf curl Burewala alphasatellite (CLCuBuA), Bhendi yellow vein mosaic virus-associated alphasatellite (BhYVMA), Mesta yellow vein mosaic virus-associated alphasatellite (MeYVMA), Cotton leaf curl Gezira alphasatellite (CLCuGA), Okra yellow crinkle Cameroon alphasatellite (OYCrCamA), Vernonia yellow vein Fujian virus alphasatellite (VYVFujA), Cotton leaf curl virus associated alphasatellite isolate Lucknow (CLCuA-Luk), Tobacco leaf curl PUSA alphasatellite (TLCuPA), Verbesina encelioides leaf curl alphasatellite (VerLCuA), Okra leaf curl disease associated alphasa- tellite (OLCuA), Gossypium mustilinum symptomless alphasatellite (GoMSA), Gossypium darwinii symptomless alphasatellite (GoDSA), Tomato leaf curl Pakistan virus associated alphasatellite (ToLCuPakA), Cotton leaf curl Multan alphasatellite (CLCuMA), Tobacco curly shoot virus associated DNA 1, Ageratum yellow vein Singapore alphasatellite (TCSA), Tomato yellow leaf curl China virus associated alphasatellite (ToYLCuCHA), Tomato leaf curl Cameroon alphasatellite (ToLCCamA), Croton yellow vein mosaic alphasatellite (CrYVMA), Papaya leaf curl alphasatellite (PLCuA), Ageratum leaf curl Cameroon alphasatellite (ALCCamA), Hollyhock yellow vein virus associated symptomless alphasatellite (HYVSA), Vernonia yellow vein Fujian virus alphasatellite (VYVFujA), Malvastrum yellow mosaic Cameroon alphasatellite (MaYMCamA) and sequence of Cotton leaf curl virus-associated Betasatellite (CLCuMB) was used as out-group sequence.

orientations. The complete nucleotide sequence the clone was determined to be 1375 nucleotides and is available in database under the accession number HF547408. Sequence analysis showed that clone as typical begomovirus complex associated alphasatellite having single gene [coordinates 70-1017] on virion sense strand that encoding single conserved Rep protein (315 amino acids). Comparison of the sequence of clone WNSX2 with sequences of alphasatellites available in databases revealed the highest level of nucleotide sequence identity with *Gossypium darwinii* symptomless alphasatellite (GoDSA; Accession no. EU384637) isolated from *darwinii* species of cotton (Nawaz-ul-Rehman et al., 2012). The highest nucleotide sequence identity was 96.7% (Table 1). The second highest nucleotide sequence identity (85.8%) was shown with Papaya leaf curl alphasatellite (PLCuA; Accession no. JQ322970). To all other alphasatellites sequences available in the databases it showed less than 70% nucleotide sequence identity. We can consider clone WNSX2 as *Gossypium darwinii* symptomless alphasatellite (GoDSA), an isolate from symptomless weed *Xanthium strumarium* and we propose the name as Gossypium darwinii symptomless alphasatellite isolate Xanthium. Its high sequence identity with Papaya leaf curl alphasatellite shows its close relation with it and on other hand Gossypium darwinii symptomless alphasatellite and Papaya leaf curl alphasatellite have 85.7% nucleotide sequence identity that why we can say WNSX2 have relation with both isolates of alphasatellite (Xu and Yoshida, 2012; Hussain et al., 2011; Amrao et al., 2010). Based on an alignment of complete genomes of selected alphasatellite from databases, a phylogenetic tree was constructed. This showed that WNSX2 forms a distinct clade with isolates of GoDSA and PLCuA (Fig. 2). The results indicate the capacity of asymptomatic plants including weeds to nurture novel as well as recombinant begomovirus associated satellites. It is very important to analyze symptomatic as well as asymptomatic plants for the presence of begomovirus and associated satellites to have the view of complete genetic diversity present in the field, so that a sustainable strategy against begomovirus can be devised.

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